ENG

Product Data Sheet:

HUMAN INTERLEUKIN-10 ELISA

Catalogue number:

RD194572200R

For research use only!

Example version

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1. INTENDED USE

The RD19457220R Human Interleukin-10 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human interleukin 10 (IL-10).

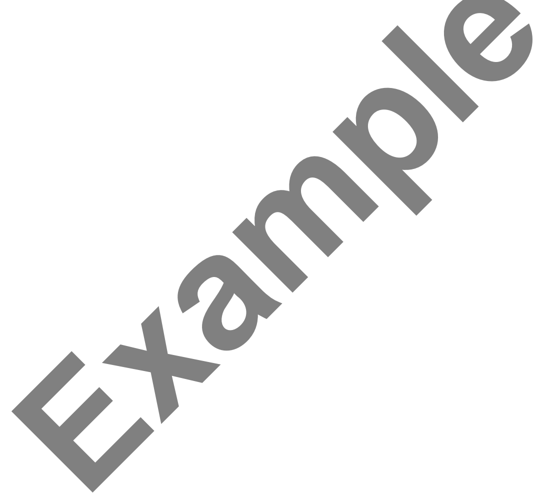
Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures IL-10 in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.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3. INTRODUCTION

Interleukin-10 (IL-10) is a Type II cytokine and the “founding” member of a family of cytokines that includes also IL-19, IL-20, IL-22, IL-24, IL-26, IL-28 and IL-29. All of these cytokines have similar intron-exon organization and bind to receptors with similar structures. IL-10 was first identified as a cytokine secreted by $CD4^+$ Th2-cells that inhibits cytokine production in antigen presenting cells, and was described as a cytokine synthesis inhibitory factor [4]. IL-10 is an important anti- inflammatory and immunosuppressive cytokine [1].

The gene for human IL-10 is located in the 1q32 band on chromosome 1 and encodes for 5 exons. The encoded protein is a homodimer with molecular mass of 37 kDa consisting of 160 amino acid monomers [4].

The list of cells producing IL-10 has expanded rapidly since its discovery, as has the number of cell types known to respond to this cytokine. Important producers of IL-10 are T-helper type 2 (Th2) cells, subset of regulatory T cells designated Tr1, Th1 and Th17 cells, $CD8^+$ T cells, monocytes and appropriately stimulated macrophages, certain subsets of dendritic cells (DCs), granulocytes including eosinophils and mast cells. Non-immune cells producing IL-10 include keratinocytes, epithelial cells and even tumor cells [7].

Expression of IL-10 in unstimulated tissues is negligible and seems to require triggering by commensal or pathogenic flora. IL-10 production is stimulated by cell activation with the bacterial endotoxin lipopolysaccharide (LPS). An initial burst of pro-inflammatory cytokines (TNFα, IL-1, IL- 6 and GM-CSF) is later followed by a rise in IL-10 synthesis [8].

The primary function of IL-10 is to down-regulate immune response and limit tissue damage [2].

IL-10 down-regulates inflammatory activation of monocyte-macrophage cells by transcriptional and post-transcriptional inhibition of the entire range of pro-inflammatory cytokines [3]. IL-10 can strongly inhibit secretion of IL-2/INF-γ by Th1 cells. It also inhibits secretion of IL-1, IL-6, IL-12, and tumor necrosis factor α in macrophages and dendritic cells in order to reduce tissue damage [1]. IL-10 may be increased by transforming growth factor β (TGFβ), interferon α (IFNα), IFNβ and histamine. On the other hand, LPS-stimulated IL-10 production may be inhibited by IL-4 and IFNγ [8].

IL-10 is a main anti-inflammatory cytokine and has been suggested to play a crucial role in neuronal homeostasis and cell survival. IL-10 plays a protective role in microglial cultures after a pro-inflammatory insult and in rat pups born to dams infected with Escherichia coli. However, several studies have questioned the perception of IL-10 solely as an immunosuppressive cytokine because it can also stimulate immune responses by promoting the proliferation and cytotoxic activity of natural killer cells and CD8+ T-cells as well as the survival, proliferation, differentiation, MHC class II expression and antibody production of B-cells. Increasing evidence indicates that IL-10 is involved in both the onset and development of inflammatory diseases [1].

IL-10 plays a pivotal role during the chronic/latent stage of pulmonary tuberculosis, with increased production playing a potentially central role in promoting reactivation of tuberculosis. IL-10 is found in serum, plasma and bronchoalveolar lavage fluid of active tuberculosis patients [2].

Increased levels of IL-10 secreted from macrophages have also been associated with certain types of cancer including prostate, breast, cervical and gastric tumors [3].

Since IL-10-mediated immune responses are quite important in maintaining intestinal homeostasis and commensal flora tolerance, it has been suggested that an aberration n IL-10 production may be involved in pathogenesis of Crohn´s disease. Impaired IL-10 production has been found in severe cases of both Crohn´s disease and ulcerative colitis [4].

Other autoimmune diseases, such as systemic lupus erythematosus, type 1 diabetes mellitus, psoriasis, and rheumatoid arthritis, have also been shown to be associated with IL-10 [4].

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Areas of investigation:

Immune Response, Infection and Inflammation

Oncology

Inflammatory Bowel Disease

Autoimmunity

4. TEST PRINCIPLE

In the BioVendor Human Interleukin-10 ELISA, standards and samples are incubated in microplate wells pre-coated with monoclonal anti-human IL-10 antibody. After 60-minute incubation and washing, biotin labelled monoclonal anti-human IL-10 antibody is added and incubated for 60 minutes with captured human IL-10. After another washing, streptavidin-HRP conjugate is added. After 30-minute incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of IL-10. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

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5. PRECAUTIONS

- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit may contain components of human or animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not been mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (100x)	concentrated	0.13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Biotin-Ab Diluent	ready to use	13 ml
Dilution Buffer	ready to use	20 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precise pipettes to deliver 5-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of shaking at approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)

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9. PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to use.

Always prepare only the appropriate quantity of reagents for your test.

Do not use components after the expiration date marked on their label.

Assay reagents supplied ready to use:

Antibody Coated Microtiter Strips

Stability and storage:

Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate

Biotin-Ab Diluent

Dilution Buffer

Substrate Solution

Stop Solution

Stability and storage:

Opened reagents are stable 3 months when stored at 2-8°C.

Example Version

Assay reagents supplied concentrated or lyophilized:

Human IL-10 Master Standard

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

The resulting concentration of IL-10 in the stock solution is **200 pg/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	200 pg/ml
250 µl of stock	250 µl	100 pg/ml
250 µl of 100 pg/ml	250 µl	50 pg/ml
250 µl of 50 pg/ml	250 µl	25 pg/ml
250 µl of 25 pg/ml	250 µl	12.5 pg/ml
250 µl of 12.5 pg/ml	250 µl	6.25 pg/ml
250 µl of 6.25 pg/ml	250 µl	3.13 pg/ml

Prepared Standards are ready to use, do not dilute them.

Stability and storage:

Do not store the reconstituted Master Standard and/or diluted standard solutions.

Example Version

Biotin Labelled Antibody Conc. (100x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (100x) to 99 parts Biotin-Ab Diluent.

Example: 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Biotin-Ab Diluent for 1 strip (8 wells). **Mix well** (not to foam).

Stability and storage:

Opened Biotin Labelled Antibody Concentrate (100x) is stable 3 months when stored at 2–8°C.

Do not store the diluted Biotin Labelled Antibody solution.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. e.g. 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage:

The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures IL-10 in serum and plasma (EDTA, citrate, heparin).

Samples can be assayed immediately after collection, or should be stored frozen. Thoroughly mix thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Serum and plasma samples:

An appropriate dilution should be assessed by the researcher prior to batch measurement.

Recommended starting dilution for serum and plasma is 3x.

Dilute samples **3x** with Dilution Buffer just prior to the assay, e.g. 50 µl of sample + 100 µl of Dilution Buffer for singlets, or preferably 100 µl of sample + 200 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:

Samples should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.

Do not store the diluted samples.

Note: It is recommended to use a precise pipette and a careful technique to perform the dilution in order to get precise results.

Example Version

11. ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Standards, Dilution Buffer (=Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Pipet **100 µl** of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Pipet **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
11. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below 20°C. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 12.**

Example Version

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine IL-10 concentration of off-scale samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 200**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	**Standard 100**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	**Standard 50**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	**Standard 25**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	**Standard 12.5**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	**Standard 6.25**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
G	**Standard 3.13**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
H	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40

Figure 1: Example of a work sheet.

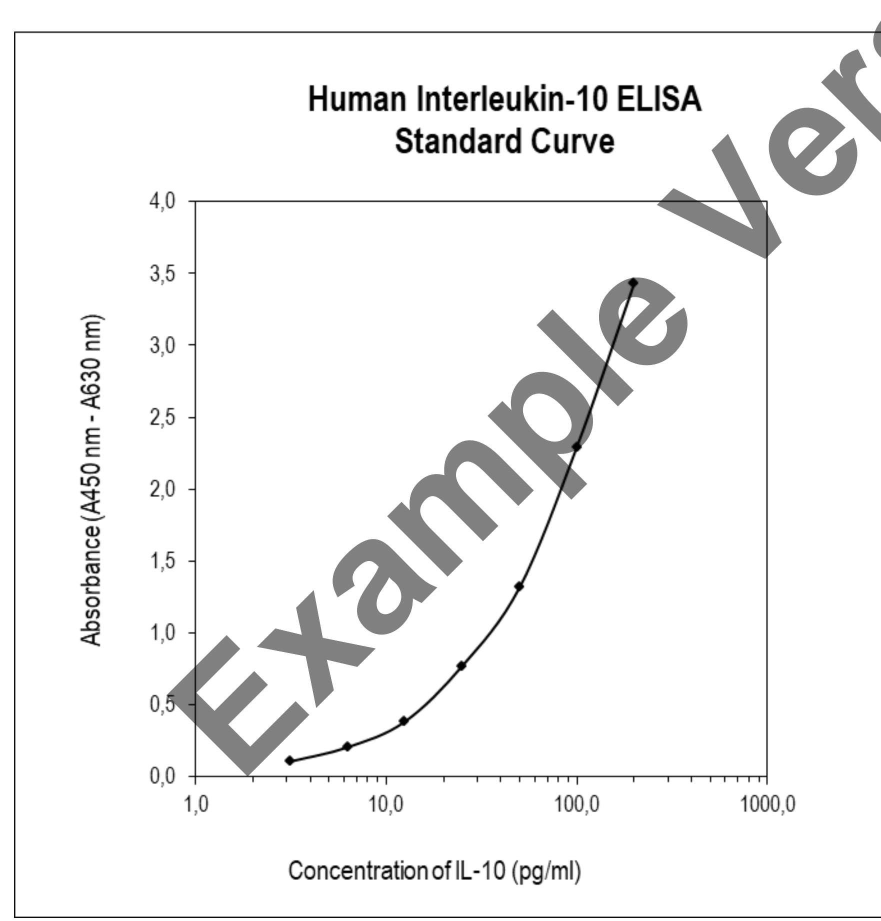
Example Version

12. CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The Standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of IL-10 (pg/ml) in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve (i.e. *logit* of absorbance (Y) is plotted against *log* of the known concentration (X) of standards).

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay - e.g. 20 pg/ml (from standard curve) x 3 (dilution factor) = 60 pg/ml.



Example Version

Figure 2: Typical Standard Curve for Human Interleukin-10 ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Interleukin-10 ELISA are presented in this chapter.

Sensitivity

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is calculated from the real IL-10 values in wells and is 1.32 pg/ml.

*Dilution Buffer is pipetted into blank wells.

Limit of assay

Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

Presented results are multiplied by respective dilution factor.

Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	26.37	0.52	2.0
2	21.78	0.41	1.9

Example Version

Inter-assay (Run-to-Run) (n=6)

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
1	23.86	1.1	4.8
2	107.33	4.0	3.7

Spiking Recovery

Serum samples were spiked with different amounts of human IL-10 and assayed.

Sample	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Serum 1	22.19	-	-
	62.37	59.69	104.5
	101.18	97.19	104.1
	173.39	172.19	100.7
Serum 2	20.74	-	-
	58.15	58.24	99.9
	93.79	95.74	98.0
	167.90	170.74	98.3

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Serum 1	-	144.41	-	-
	2x	63.38	72.21	87.8
	4x	31.52	36.10	87.3
	8x	18.10	18.05	100.3
Serum 2	-	265.60	-	-
	2x	136.06	132.80	102.5
	4x	65.72	66.40	99.0
	8x	34.92	33.20	105.2

14. DEFINITION OF THE STANDARD

The Standard used in this kit is a recombinant protein. The recombinant human IL-10, produced in *E. coli*, is an 18.647 kDa protein consisting of 160 amino acid residues.

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15. PRELIMINARY POPULATION AND CLINICAL DATA

Fifty serum samples from unselected healthy donors (32 men + 18 women) were assayed with the BioVendor Human Interleukin-10 ELISA in our laboratory. All of them were measured under the lowest standard (3.13 pg/ml).

Reference range

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological references ranges for IL-10 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Interleukin-10 ELISA has not been compared to any other commercial immunoassay.

Example Version

17. TROUBLESHOOTING AND FAQS

Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper manual washing
- Improper wavelength when reading absorbance

High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards or samples

18. REFERENCES

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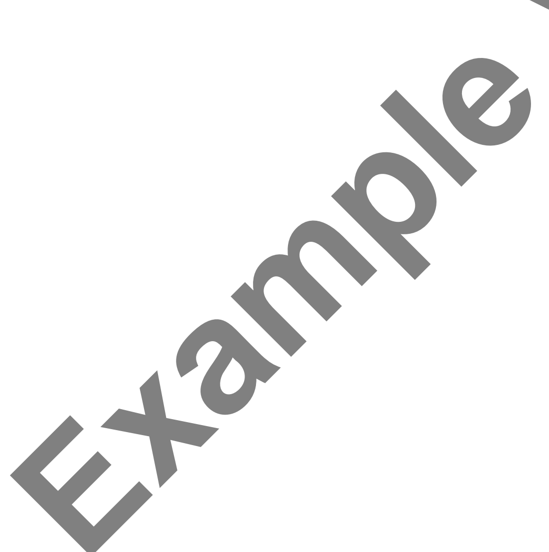
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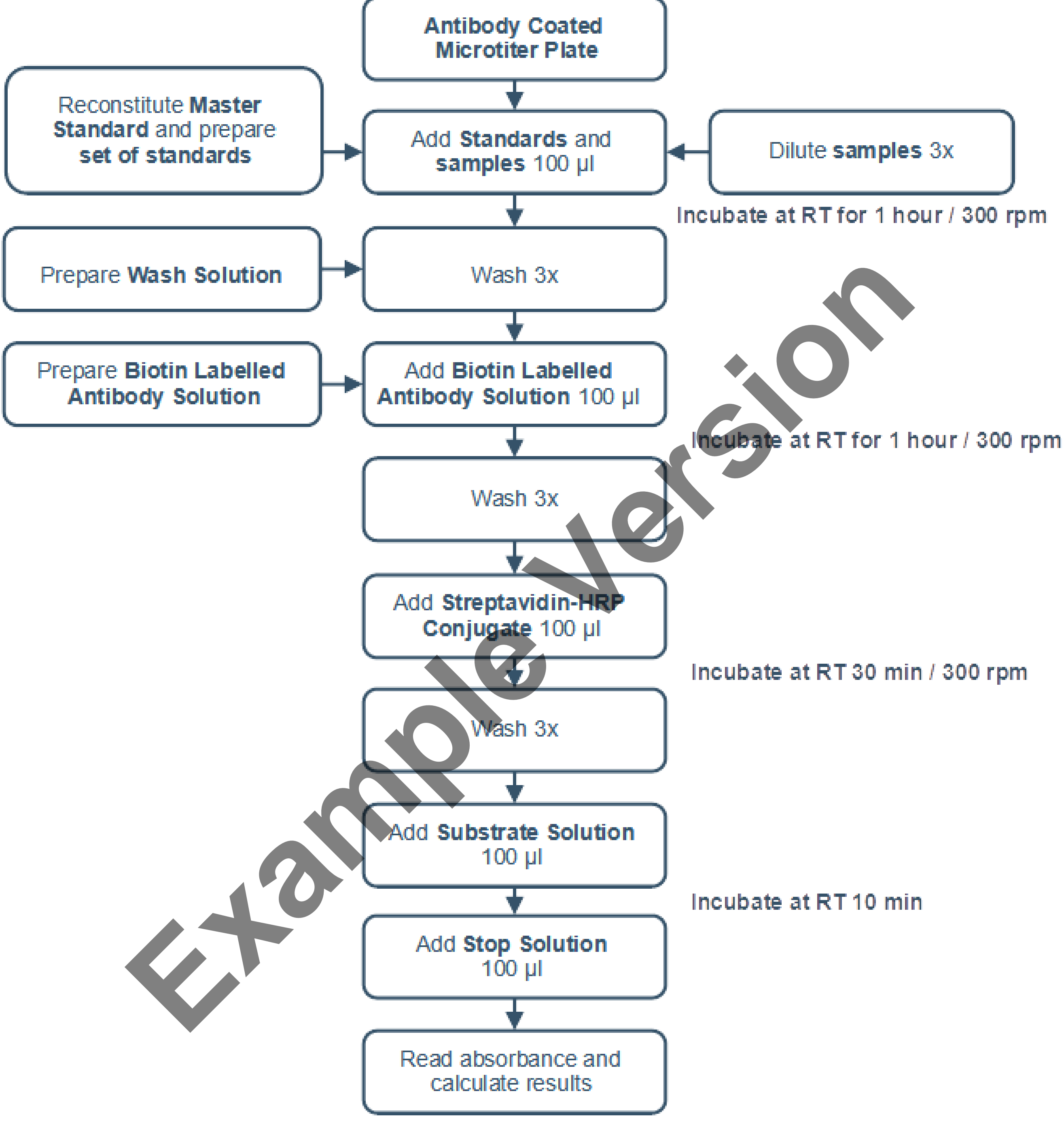
19. EXPLANATION OF SYMBOLS

Symbol	Meaning
REF	Catalogue number
LOT	Batch code
	Caution
	Use by date
2 °C 8 °C	Temperature limit
	Manufacturer
www.biovendor.com	Read electronic instructions for use - eIFU
96	The content is sufficient for 96 tests
	Biological risks

Example Version

20. ASSAY PROCEDURE - SUMMARY



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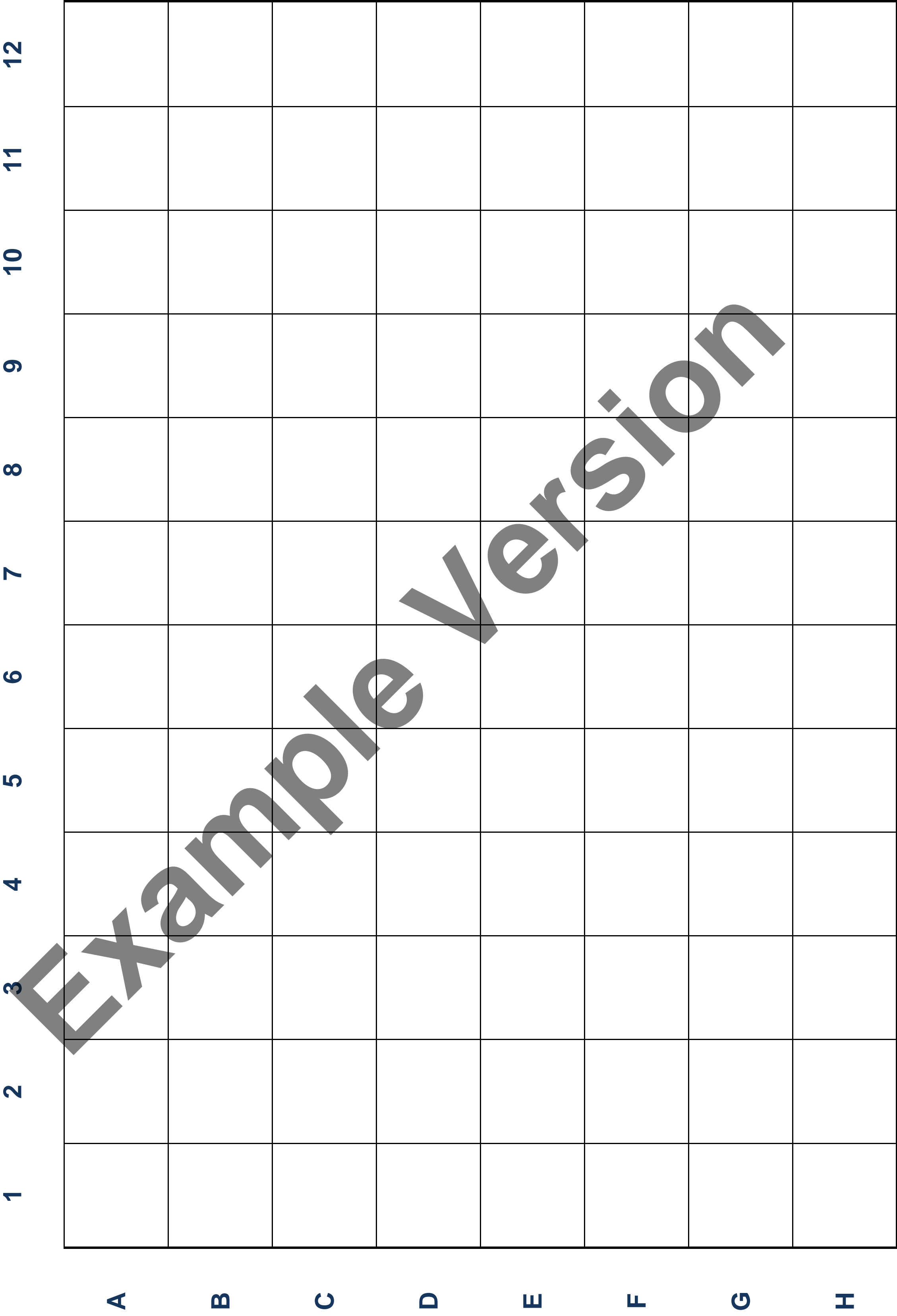

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